

Mapping the Way to Bovine Bounty

Like road crews installing signposts on a long stretch of a highway, animal researchers are marking off regions of bovine DNA that will point the way to genes for a plethora of traits—from ideal body shape, ease of calving, and disease resistance, to protein-rich milk for dairy products.

And by inspecting the chromosomal housing for such DNA researchers aspire to use new means of predicting the inheritance of traits in offspring sired by prized bulls, or, by their semen samples, for that matter.

Yet, even with the artificial insemination of cows with such bull semen, dairy breeders must wait about 5 years before they can find out whether a bull calf will bear all the desired traits, says Melissa S. Ashwell. She's an animal geneticist at the ARS Gene Evaluation and Mapping Laboratory in Beltsville, Maryland. Her colleagues there are ARS geneticists Tad S. Sonstegard and Curtis P. Van Tassell.

With marker-assisted selection, a technique that would use the team's mapping information, timely genetic forecasts might be made from just a few embryo cells at a diagnostic lab, or using blood drawn from a newborn calf.

"If we had a particular marker, we should be able to predict from the embryo whether the desirable traits will be expressed. And even if you have to wait until that bull calf is born, you'd still save a lot of time and money versus waiting 5 years to see if the calf has the genetic potential to produce desirable dairy cows," says Ashwell.

Since the mid-1990s, her team has used mapping techniques to examine 150 to 200 different DNA markers from the semen of stud bulls—or sires—linked to 8 Holstein families. Each of these 8 patriarch bulls has sired at least 75 to 250 sons. The sons, in turn, fathered no less than 50 daughters.

Why the focus on Holsteins? "Their high milk production makes them the primary breed in the dairy industry," says Ashwell.

Marking a Territory—of DNA

For Ashwell and her co-workers, the search for important genes begins with an assortment of high-tech tools, including a high-throughput sequencer, and special probes called oligonucleotide primers. The latter finds and binds to genetic pieces of interest. Another tool is PCR. It's like a xerox machine that generates millions of copies of these DNA pieces—including marker regions—so they can be identified and sequenced.

"A marker is a very short stretch of DNA, probably 100 to 200 nucleotides long," Ashwell explains. It helps if you think of DNA as the alphabet that spells out an organism's traits and innermost workings. The nucleotides, then, are the chemical letters making up that alphabet. "The differences you see in markers are sequence differences in nucleotides," she says.

Using this technology, the Beltsville team in 1995 was first to stake a claim on a region of chromosome 23 where genes

PEGGY GREB (K8927-1)



Animal geneticist Melissa Ashwell removes bull semen from a liquid nitrogen storage tank. DNA will be extracted from the semen and used for marker analysis.

may mediate a cow's response to mastitis. Susceptible animals can acquire this costly disease through *Staphylococcus* and other bacterial infections of their udder.

Dairy producers lose an estimated \$2 billion annually to the disease. Ideally, with marker-assisted selection techniques, commercial breeders could select for resistance traits based on such indicators as the animals' somatic (white blood) cell scores. This refers to the concentration of cells per milliliter of cow's milk. Ashwell cautions they're still uncertain how "statistically significant" the finding is and says it needs to be studied more in contemporary animals.

In the meantime, the team has taken their molecular prospecting to other chromosome regions. One of those, located on chromosome 27, is associated with "dairy form." This trait

PEGGY GREB (K8926-1)



Geneticist Tad Sonstegard prepares to load PCR samples onto an automated DNA sequencer.

SCOTT BAUER (K7963-1)



Information gained from mapping the genes of cows will one day be used to select dairy cows with greater milk production and improved disease resistance.

describes a cow's physical appearance, and may also be an indicator of ketosis, a metabolic disorder that typically affects cows with newborn calves. For ketosis, the scientists' goal is to come up with a fast, accurate method of identifying animals less likely to suffer the disorder.

Determining Traits Expressed by Genes

Whether dairy form or ketosis, the challenge for Ashwell and her colleagues is linking what's seen on chromosomes in the lab with physical evidence in the animals. In some cases, the team's findings run parallel to those of other researchers. At Pennsylvania State University, for example, geneticist Gary Rogers is investigating relationships among measures for body fat deposition and incidence of disease in dairy cows. Farther

west, at ARS' U.S. Meat Animal Research Center in Nebraska, geneticist Eduardo Casas is focusing attention on marbling traits in beef carcasses. Marbling affects the tenderness of meat cuts like steak and is associated with an animal's fat content. Based on this, Rogers' data, and marker work at Beltsville, "we think the same gene or gene cluster could be involved in marbling in beef cattle and fat content in dairy cows," reports Ashwell.

She is quick to credit the ongoing work of human genome mappers for helping them blaze the trail in bovine studies. Much of that's because animal researchers can extrapolate data from human genome mapping to zero-in on the equivalent on cow chromosomes.

For example, "using fluorescent tags, we use markers from bovine chromosome 27 (there are 29 total in cattle, plus the two sex chromosomes, X and Y) and see what region that corresponds to in human chromosomes," says Ashwell. "So much is known about human chromosomes—we try to feed off that knowledge and identify candidate genes that may explain the differences in dairy cattle."

But selecting these candidate genes isn't always easy. For example, the Beltsville team recently identified an association between DNA markers on chromosome 6 and the amount of protein in milk. Many of these milk proteins are called caseins.

"These genes would be obvious candidate genes that could have explained the differences we see in protein content," Ashwell says. "But the casein genes are located close to the chromosome's bottom, and we're seeing our effect at a different location on the chromosome much further up. This suggests that there are several different genes that affect important traits like protein content."

Eventually, information gleaned from this genetic riddle may offer a way for breeders to select animals whose milk contains even more protein. Casein proteins are especially important for cheesemaking. But some caseins are better suited than others to this. With the right genetic tools, Ashwell ventures, there may come a day when cows are bred for milk that contains specific proportions of these cheese-friendly proteins.

By marking off chromosome regions, scientists can begin creating a genetic roadmap that can be used to put desirable traits into cows. "If we can get this mapping technology perfected," says Ashwell, "we're going to breed better and better cows."—By **Jan Suszkiw**, ARS.

This research is part of Animal Genomes, Germplasm, Reproduction, and Development, an ARS National Program (#101) described on the World Wide Web at <http://www.nps.ars.usda.gov/programs/appvs.htm>.

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